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Photodynamic Therapy and Hyperthermia as an Adjuvant Modality in Preventing Tumor Recurrence

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Background and Objective: **The objective of this study was to determine the relative efficacy in preventing tumor recurrence by photodynamic therapy (PDT), and by ablative CO_2 laser surgery followed by PDT, compared to ablative surgery alone (negative control) or ablative surgery followed by a course of hyperthermia (positive control).**

Study Design/Materials and Methods: **The cheek pouches of 36 hamsters were treated with 0.5% 9,10 dimethyl-1,2-benzanthracene in acetone three times a week. After 12 weeks all animals showed tumors of their cheek pouches and were divided into four groups. In groups number I, II, and III, all visible tumors were removed by aid of a CO_2 laser. Animals of group I did not receive any further treatment. After tumor resection, the cheek pouches in group II were treated with hyperthermia by aid of a Nd:YAG laser and a temperature of 43°C for 30 minutes. In group III after resection of the tumors, the cheek pouches were treated with PDT (75mW/cm² 175J/cm²—3mg/kg Photofrin i.p./ 24h). In group IV, the tumors were not excised, instead they were only treated with PDT (as above). All animals were observed for 50 days for any signs of tumor recurrence.**

Results: **In group I (CO_2) all tumors (100%) recurred within 50 days. In group II (CO_2 + hyperthermia) 61%, in group III (CO_2 + PDT) 27.7%, and in group IV (PDT) 50% of all tumors recurred. The first signs of recurrence could be seen in group I, followed by groups II and IV. Group III was the last one presenting tumor recurrence.**

Conclusions: **The combination of CO_2 surgery and PDT produced significantly better results than CO_2 surgery or PDT alone, and better than the combination of CO_2 surgery and hyperthermia. Lasers Surg. Med. 20:188–194, 1997.** © 1997 Wiley-Liss, Inc.

Key words: photodynamic therapy; tumor recurrence; CO_2 laser; hamster cheek pouch

INTRODUCTION

Tumors of the head and neck area are primarily squamous cell carcinomas which grow rapidly and metastasize early to the regional lymph nodes [1]. One of the major problems for the clinician dealing with these tumors is the high rate of local tumor recurrence which depends on several factors like tumor size, lymph node metasta-

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sis, and the form of therapy used for treatment. Today, mainly extensive surgery, and the combination of surgery, radiotherapy, and chemotherapy are used to treat these tumors.

In the head and neck, photodynamic therapy (PDT) has shown encouraging results when used on superficial or small tumors [2–4]. For the treatment of larger tumors, PDT was initially used as a palliative form of therapy. Such tumors have been treated with multiple interstitial fiber implantations in combination with superficial illumination. Due to the large tumor mass and the difficult light distribution in the upper aerodigestive tract, curative results have been obtained. According to these results, PDT can only serve as a palliative form of therapy [5].

A new point of view for the application of PDT in the treatment of large and more advanced tumors could be the combination of surgery and PDT. After tumor resection, the tumor bed would be treated by PDT in order to destroy any residual tumor or premalignant cells.

As photosensitizers are retained rather selectively in malignant cells, PDT fulfills most of the criteria for selective intraoperative tumor therapy [5]. Activation of the sensitizer with light of the proper wavelength and appropriate energy level causes the irreversible oxidation of intracellular components by singlet oxygen, which causes cell death of the residual malignant cells. Beside this direct cell targeting effect, PDT also has a vascular effect. The activated photosensitizer causes vascular endothelial damage with vascular shutdown and erythrocyte leakage followed by ischemic tumor necrosis [6].

Hyperthermia has proven efficacious in the treatment of certain human malignancies. This has been demonstrated using hyperthermia alone or in combination with radio- or chemotherapy [7]. Earlier reports have demonstrated that the combination of hyperthermia and PDT is effective in the treatment of certain tumors [8,9].

The objective of this study was to determine the relative efficacy of preventing tumor recurrence by PDT, and by ablative CO_2 laser surgery followed by PDT, compared to ablative surgery alone (negative control) or ablative surgery followed by a course of hyperthermia (positive control).

MATERIALS AND METHODS

Drugs/Compounds

Photofrin was obtained as a freeze-dried powder from Quadra Logic Technology Phototherapeutics, Inc. (Vancouver, BC, Canada). The powder was reconstituted in sterile 5% dextrose in water for a final concentration of 2.5mg/cc. 9,10 Dimethyl-1,2-benzanthracene (DMBA) was obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in acetone to produce a 0.5% solution.

Animal Model

Syrian Golden Hamster (*Mesocricetus auratus*) retired breeders (200g) were obtained from Charles River Laboratories (Kingston, NY).

Both pouches, in each hamster, were painted with 0.5% DMBA in acetone three times per week for 12–14 weeks. Studies have shown that after 10–12 weeks of topical application, papillary and frankly invasive carcinomas appear and after 12–14 weeks, extensive tumors with invasion and surface necrosis occurred. In the present study, retired breeders were used as these developed cancer more rapidly and reproducible than younger animals. The hamsters were housed in single cages, fed with pelleted laboratory diet food and given water ad libitum. The hamsters were anesthetized with ether. This facilitated application in an orderly and repeatable fashion, and minimized animal discomfort. The buccal mucosa of both pouches was exverted and a 3–4 cm^2 area painted three times per week with 0.5% DMBA in acetone solution using a no. 4 sable brush. Acetone was used rather than mineral oil as a vehicle, because it dried on the localized area. By this localized application method, tumor occurred only on the buccal mucosa and not all over the oral cavity. After 12–14 weeks of continuous DMBA application, all animals exhibited gross papillary tumors ranging from one to six per pouch.

Laser Systems

Argon-dye. Six hundred and thirty nanometers of light was delivered using a Spectra-Physics argon (model 375) dye (model 164) laser system (Spectra Physics, Mountain View, CA). The 630 nm light was obtained from the tunable dye laser using Kiton red dye. The beam from the dye laser was coupled to a 400 micron quartz fiberoptic with a diverging lens attached to the end to facilitate a uniform distribution of the light over the treatment field.

Nd:YAG. Hyperthermic induction was obtained using a continuous wave Nd:YAG laser (Quantronix, Smithtown, NY, Model 114) emitting light at 1,060 nm. The light was delivered via a 400 micron quartz fiberoptic with a diverging

lens attached in the same manner as the 630 nm light. Temperature measurements were accomplished by placing copper-constantan microthermocouples contained within 30-guage stainless steel needles (Omega Corporation, Stamford, CT) directly into the tumor.

Carbon dioxide. Tumor excision was accomplished by use of a CO_2 laser (Cooper, Model 870). This system was used in the continuous wave mode using a power output of 2 watts maximum.

Treatment Modalities

Animals were randomized into four treatment groups. Each group consisted of nine animals (18 pouches). For treatment, the animals were anesthetized by using pentobarbital i.p.

Group I: CO_2 laser excision. After anesthesia, the cheek pouches were everted and all visible tumor and all suspicious mucosa were excised by a CO_2 laser (Cooper, Model 870). The CO_2 laser was running in a continuous wave mode with 2 watts power output. The resected tumors and mucosa were fixed in buffered formalin and sent for histopathological analysis. After tumor resection, the animals were replaced into their cages for recovery.

Group II: CO_2 laser excision + hyperthermia. Animals in group II underwent the same treatment as animals in group I. All visible tumor and dysplastic mucosa were resected by aid of the CO_2 laser. Twenty-four hours after the tumor resection, the animals were anesthetized again. Their cheek pouches were everted and two copper-containing microthermocouples within 30-gauge stainless needles were placed in the mucosa of each pouch for temperature monitoring.

Hyperthermic induction was obtained using continuous wave Nd:YAG laser (Quantronix, Smithtown, NY, Model 11) emitting at 1060nm. The temperature used during the treatment was regulated by aid of the thermocouples. Within 5–10 minutes, a temperature of 43.0°C was reached. This temperature ±0.5°C was maintained for an additional 30 minutes. Following the hyperthermia therapy, the animals were replaced into their cages for recovery.

Group III: CO_2 laser excision + PDT. Animals in group III were treated like animals in group I. All visible tumor and suspicious mucosa was excised by aid of a CO_2 laser. After tumor resection, all animals were given 3mg/kg Photofrin i.p. Twenty-four hours later, the animals were anesthetized again. Their pouches were again everted. The whole buccal mucosa was illuminated superficially with 630nm light at 75mW/cm^2 and 175J/cm^2 delivered by the argon-dye laser system. After PDT, the animals were replaced into their cages for recovery.

Group IV: PDT alone. Animals in group IV were given 3mg/kg Photofrin i.p. Twenty-four hours later (no CO_2 laser tumor resection), their cheek pouches were everted and a biopsy for histological analysis was taken.

The whole cheek pouch including all dysplastic mucosa, all tumors, and also the normal appearing mucosa was illuminated superficially with light at 630nm. Light was delivered by an argon ion pumped dye laser at a power density of 75mW/cm^2 and a dose rate of 175J/cm^2, as previously described. After PDT, the animals were placed into their cages for recovery.

All animals were maintained under normal feeding and care conditions. The buccal cheek pouches were checked every other day until regrowth was observed or until 50 days had passed for the purpose of determining long-term cures and interval of tumor recurrence.

Histological Diagnosis

The biopsies were placed in 10% formalin for routine histological preparation and hematoxylin and eosin (H&E) staining. Histologic examination for presence of invasive squamous cell carcinoma, carcinoma in situ, severe dysplasia, moderate dysplasia, mild dysplasia, hyperkeratosis, or other abnormality was performed on each buccal mucosa by a pathologist blinded to the treatment modalities.

Statistics

P-values were calculated by Mann-Whitney Test.

RESULTS

Lesion Development

After 12 weeks of DMBA application, macroscopically all hamsters presented several exophytic growing tumors (one to six tumors each pouch) of the buccal mucosa. Tumors were limited to the buccal mucosa; no other localization, such as in the palate or tongue, appeared. Histological analysis of the biopsies showed that only 10% of all clinical tumors corresponded to invasive squamous cell carcinomas. Most of the biopsies showed severe (30.5%) or moderate (29.2%) dysplasia. Detailed information on the histological grading

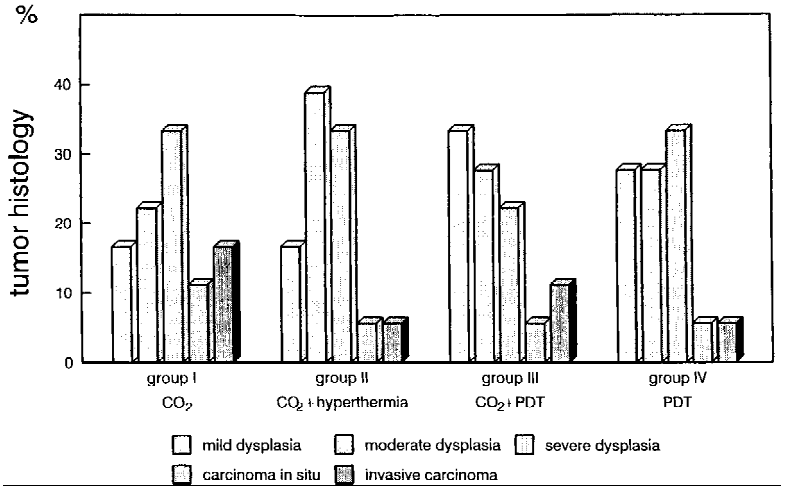

Fig. 1. Histological grading within each treatment group.

within the treatment groups is shown in Figure 1. Tumor size ranged between 2–6 mm.

Immediate Response to Treatment

After CO_2 laser excision, all tumors and dysplastic mucosa were removed, leaving slight scars behind. Hyperthermia treatment after CO_2 laser excision (group II) caused localized necrosis of the buccal mucosa. This necrosis did not occupy the complete irradiated field. The necrosis appeared within 2–4 days after hyperthermia treatment and healed within several days. There was residual scar formation after healing was complete.

PDT treatment after CO_2 laser excision also caused localized necrosis of the buccal mucosa, similar to the findings after hyperthermia treatment. Necrosis of the mucosa appeared within 2 days after PDT treatment.

Animals of group IV, receiving no CO_2 laser excision, developed a more widespread necrosis than all other animals. Within 48 hours after PDT treatment, all tumors as well as wide areas of the DMBA treated buccal mucosa became necrotic.

Treatment Effects on Lesion Recurrence

In the CO_2 laser group (group I) all animals developed tumor recurrence of both cheeks within 50 days (18/18 cheeks). First signs of clinical tumor recurrence were seen after 7 days. Within 40 days, all animals showed tumor recurrence (Figs. 2, 3).

Animals in group II (CO_2 + hyperthermia) developed tumor recurrence significantly slower than animals treated only by CO_2 laser excision ($P<0.01$). Within 50 days, only in 61% of all cheeks (11/18 cheeks), tumor recurred (Figs. 2, 3).

Tumor recurrence in group III (CO_2 + PDT) was the lowest of all treatment groups. Only 27.7% of all cheeks (5/18 cheeks) showed any sign of tumor recurrence within the follow-up of 50 days after therapy. Therefore tumor recurrence was significantly lower than after CO_2 laser excision ($P<0.01$). Tumors of this group recurred slowest of all groups (Figs. 2, 3).

For the animals treated exclusively by PDT treatment, 50% of all cheeks (9/18 cheeks) showed tumor recurrence within the follow-up of 50 days. Tumor recurrence was significantly slower than for animals in group I ($P<0.03$). Tumors recurred overall, at a rate similar to groups II and III (Figs. 2, 3).

Tumor growth after tumor recurrence was rather difficult to quantify, but there was evidence of promoted tumor growth in the group of exclusive CO_2 laser excision, compared to all other groups. Tumors in this group grew much faster and larger than any other treatment group.

DISCUSSION

The current study examined the rate of tumor recurrence after surgical tumor removal and different adjuvant treatment modalities.

CO_2 laser surgery was used for tumor resection as laser surgery has a major role in the present and future health care system. Laser surgery is useful and often superior to the scalpel in difficult to access areas like the oral cavity, nasal, pharynx, and oropharynx. CO_2 laser has advan-

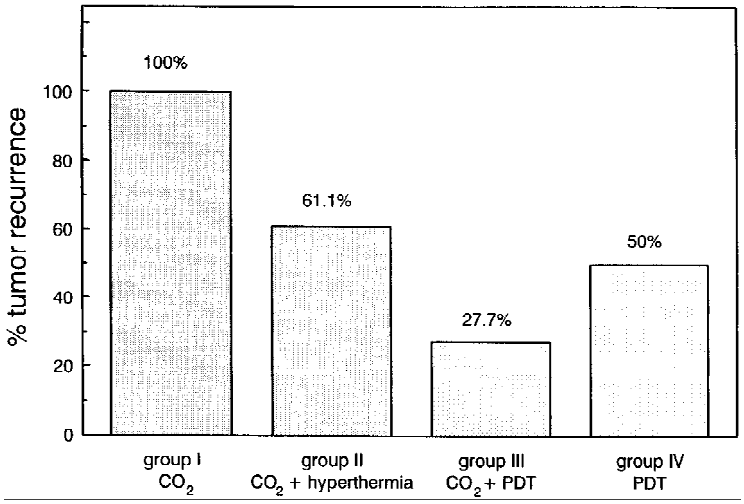

Fig. 2. Rate of tumor recurrence within 50 days after therapy for different treatment groups.

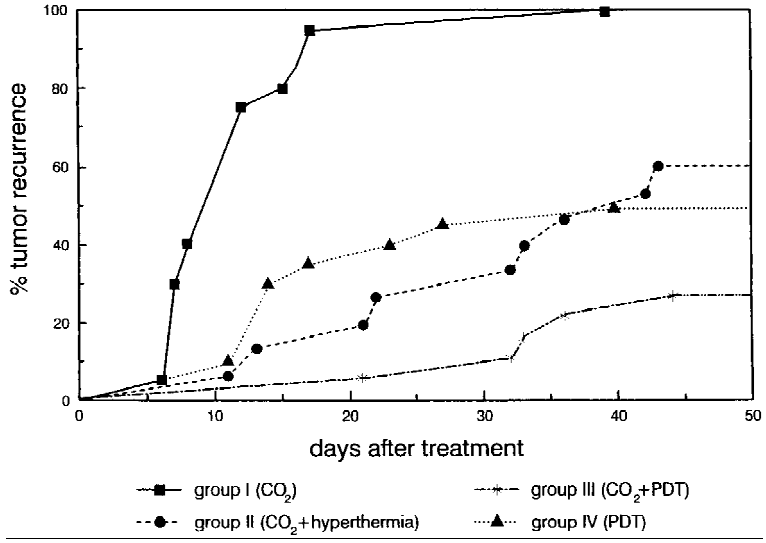

Fig. 3. Time interval for tumor recurrence for each treatment group.

tages of decreased patient discomfort, less postoperative scarring, and increased rate of re-epithelization.

The animal model that we have used was the dysplasia or squamous cell carcinoma produced in the hamster buccal cheek pouch by DMBA application. After development by Salley in 1954, the model was studied more extensively by Morris et al., and others [10,11]. Since then, it has proved to be a reasonable example of well-differentiated epidermoid carcinoma and a model for the human oral cancer situation. Therefore, it should serve well as a model for studies of different treatment modalities of developing oral cancer. These observations could also serve as a good model for epithelial cancers developing in response to field cancerization of other sites as well. DMBA is a complete carcinogen (initiator and promotor). Therefore, a repeated application of DMBA causes a consistent sequence of histological changes to transpire in the affected pouch mucosa. After 12–14 weeks of continuous application, we found several stages of dysplastic transformation ranging from mild dysplasia to invasive cancer [19].

It is important to recognize that different histopathological grading could be found in the same treated cheek pouch at the time of examination. Therefore, a variability in the degree of epithelial transformation within and between the animals exists. The biopsies we took could only be

representative for one location. The concept of field cancerization is integral in this observation. The aspect of field cancerization seems to be important in order to explain the high rate of 100% tumor recurrence in the group treated only with CO_2 laser surgery. This high rate of tumor recurrence clearly indicates that by the present surgical method, we did not resect all condemned or dysplastic tissue which appeared to be normal. This condemned or dysplastic tissue with already initiated cancer was probably the origin for later tumor recurrence.

The time scale and growth tendency of recurring tumors within the CO_2 laser group seem to be remarkable. After CO_2 laser excision, tumors arose significantly earlier and grew much faster than in any other treatment group. The greater growth potential of tissue after CO_2 laser wounding was already described by other authors [12, 13]. They found a significantly higher release of epidermal growth factor as well as nerve growth factor after CO_2 laser wounding than after scalpel surgery. This was consistent with the increased healing rate and occasional excessive growth of tissue seen after CO_2 laser surgery in the oral cavity.

A review of the literature reveals only one single case report of malignant change in oral premalignant lesions treated with the CO_2 laser [14]. The authors supposed the physical stimulus of the CO_2 laser as a promotor, like several studies, has demonstrated the promoting effect of the cryoprobe to malignant changes [15,16].

Previous studies have demonstrated the potentiating effect of photodynamic therapy and hyperthermia in combination tumor therapy. These studies have demonstrated a time and sequence dependence on the extent of the interaction betweeen PDT and hyperthermia [9,17], but little data is available about the combination of surgery and PDT or surgery and hyperthermia. Sieg et al. demonstrated some potentiation of surgical tumor resection by photodynamic therapy in the mouse model [18]. A dose dependence of the interaction between surgery and PDT was observed in that study.

The data presented in this study suggest advantages in the combination of surgery and PDT compared to: a) surgical tumor resection; b) PDT as a single modality; or c) the combination of surgery and hyperthermia. A significant reduction in tumor recurrence by the combination of CO_2 laser surgery and post-operative photodynamic therapy, as well as by the combination of CO_2 laser surgery and hyperthermia, has been demonstrated. Besides the total reduction of tumor recurrence, an extension of the tumor free interval for both treatment combinations is notable. As shown in group IV, single PDT treatment could also cause a significant reduction of tumor recurrence. The selective necrosis of premalignant and malignant lesions within the entire DMBA treated field in the cheek pouch after PDT might serve as an explanation for the potentiating effect of surgery and PDT. The ability, therefore, to use PDT for the treatment of field disease becomes plausible.

It is possible, that by optimizing the photodynamic dose (light + drug dose), the hyperthermic dose (time and/or temperature) or changing the time frame or sequence in the administration of adjuvant therapy, the rates for tumor recurrence and tumor free intervals could be further improved.

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